Exh. D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Nelson, J. Group: 1614

Serial No. 09/615,639 Examiner: Sarada Prasad

Filed: July 13, 2000

For: COMPOSITIONS AND METHODS FOR THE TREATMENT OF PARKINSON'S DISEASE

DECLARATION OF PATRICIA L. STRANAHAN, M.D., Ph.D.

I, Patricia L. Stranahan, M.D., Ph.D. state as follows:

I am Medical Director of Alpha Research Group, and have been employed in that position since March 22, 2000. I am presently Professor of Pathology at Ross University School of Medicine, having recently accepted this appointment last year (2001). Prior to this appointment, I served as Professor and the Chair of the Department of Biology at Metropolitan State College of Denver (1988-2000). I have held both Regular and Adjunct Fellow/Assistant Professorships at the University of Colorado Health Sciences Center where I taught Pathology, Physiology and Biophysics, Histology and Pathophysiology (1985-1999). Prior to becoming a Professor of Pathology, I served as a Pathologist in both military and civilian capacities (1978-1984). I am double board certified in Pathology, both Anatomic and Clinical (ASCP). I am Board eligible (ASCP) in Blood Banking and Hematopathology. A copy of my curriculum vitae is attached hereto.

I have reviewed Patent No. 5,430,039 and the claims pending in the above-captioned application. It is my opinion that the pending claims are not obvious in view of the said patent because the '039 patent does not enable one skilled in the art of medicine to treat an ischemic event (stroke, CVA) using chloroquine. In fact, '039 describes a treatment approach that would be detrimental to administer following the incidence of ischemia, neuronal or otherwise, by persons skilled in the art and knowledgeable in the pathological cascades precipitated by ischemic events for the following reasons:

As is known to the art, cerebral ischemia immediately triggers an inflammatory cascade in which cytokines, tumor necrosis factor alpha (TNF-α) and interleukins (IL), the most relevant to the topic at hand being IL-6, are released. TNF and IL-6 degrade inhibitor κB-α (I-κB-α), which prevents the activation of nuclear factor κB (NF-κB). Once activated, NF-κB migrates to the

nucleus and augments mRNA synthesis of other "mediator compounds" that contribute to and/or participate in the body's inflammatory response. One such "mediator" that is produced by NF-κB activation is inducible nitric oxide synthase (iNOS), which increases nitric oxide and nitrite oxide (NO and NO_2, respectively) formation in ischemic tissues. Furthermore, NF-kappa-B activation augments the production of other reactive oxygen species, such as superoxide radical formation (O_2^-). Oxygen free radicals combine with the newly formed and abundantly available reactive nitrogen intermediates (i.e., NO and NO_2) to generate peroxynitrite ($ONOO^-$), which results in extensive neural damage following an ischemic event. Any cell producing high levels of NO and/or $ONOO^-$ will inhibit its own respiration and that of surrounding cells (see, e.g. Brown, G. and Borutaite, V., (2000), "Nitric oxide, cytochrome c and mitochondria," Biochemical Society Symposium 66:17-25).

The tissue damage is exacerbated when reperfusion injuries occur (as happens in 50% of cases of Ischemia, as discussed above), because reperfusion results in surges of NO and O_2^- generation, to produce $ONOO^-$ which mediates a predominant amount of reperfusion damage.

After NF-κB has unregulated the synthesis of the inflammatory response mediators, it eventually induces the synthesis of mRNA required to produce the inhibitor molecule I-κB-α. Once I-κB-α is then re-synthesized, it binds directly to and inhibits NF-κB. The inflammatory response and production of reactive species will then begin to damp down, however, generally not before a great deal of neural damage has been done. See Trajkovic, V., et al. (2001), "Amphotericin B potentiates the activation of inducible nitric oxide synthase and causes nitric oxide-dependent mitochondrial dysfunction in cytokine-treated rodent astrocytes," GLIA 35(3):180-188; Ichiyama, T. et al. (2001), "Thiopental inhibits NF-kappaB activation in human glioma cells and experimental brain inflammation," Brain Research 911(1):56-61; Jarosinski, K.W., et al. (2001), "Specific deficiency in nuclear factor-kappaB activation in neurons of the central nervous system," Laboratory Investigation 81(9):1275-1288; Sekine, N. et al. (2001), "GH inhibits interferon-gamma-induced signal transducer and activator of transcription-1 activation and expression of the inducible isoform of nitric oxide synthase in INS-1 cells," Endocrinology 142(9):3909-3916; and Ganster, R.W., et al. (2001), "Complex regulation of human inducible nitric oxide synthase gene transcription by Stat 1 and NF-kappa B," PNAS US 98(15):8638-8643).

It is well known to those skilled in the art that chloroquine is a potent inhibitor of both TNF-α and IL-6. See Park, Y.C., et al. (1999), "Chloroquine inhibits inducible nitric oxide synthase expression in murine peritoneal macrophages," Pharmacology & Toxicology 85(4):188-191; Weber, S.M. and Levitz, S.M. (2000), "Chloroquine interferes with lipopolysaccharide-induced TNF-alpha gene expression by a nonlysosomotropic mechanism," Journal of Immunology 165(3):534-1540; Harabak, A. et al. (1998), "Action of chloroquine on nitric oxide production and parasite killing by macrophages," European Journal of Pharmacology 354(1):83-90. In that both TNF-α and IL-6 "initiate" the inflammatory cascade by degrading the NF-κB inhibitor compound, I-κB-α, it is reasonable to assume that chloroquine and similar agents do in fact possess neural protective properties suitable to be employed for ischemia and other noxious

events—which is exactly what patent '039 demonstrates. However, the teachings of this patent do not enable one skilled in the art to use chloroquine to treat an ischemic event in a patient.

Examples in the '039 patent show treatment of brain tissue with mepacrine *prior to or at the time of* damaging the tissue with kainate or tying off blood vessels to simulate cerebral ischemia (the patent asserts that chloroquine can be substituted for mepacrine). See col. 3, line 67 through col. 4, line 2: "In FIG. 1 cannulated rats received 160 nmol of mepacrine (cross hatched bar), or vehicle (solid bar), by *icv* infusion, 10 minutes *prior* to and 3 hours following icv infusion of kainic acid." See also col. 4, lines 11-14: "In FIG. 2 cannulated rats received 160 nmol of mepacrine (cross-hatched bar), or vehicle (solid bar), by icv infusion, immediately prior to icv infusion of kainic acid." At col. 6, lines 59-63: "As shown in FIG. 7, gerbils received mepacrine (80 mg/kg, ip) (cross hatched bar), or vehicle (control) (solid bar), *immediately prior to* and once a day (40 mg/kg, ip) for 6 days after bilateral occlusion of the carotid arteries." When spectrin breakdown was stimulated by NMDA, mepacrine and chloroquine were co-administered with NMDA or administered immediately afterward (col. 6, lines 1-21). After traumatic transection of the fimbria-fornix, mepacrine was administered mepacrine *at the time of* transection.

Thus, as is demonstrated in '039, if administered prior to the beginning of the cytokine cascade initiated by a cerebral ischemic event, chloroquine prevents degradation of I-κB-α, which inhibits NF-κB and prevents formation of the reactive species that are so damaging to neurons.

However, if chloroquine is administered after the ischemic event, when cytokine production has been initiated (which happens immediately) it prevents synthesis of I-κB-α, thus allowing for enhanced, unchecked, prolonged activation of NF-κB, which would serve to enhance production of damaging reactive species. Thus, when administered following the initiation of the inflammatory cascade, chloroquine soon potentiates the availability of noxious oxygenated and nitrogen radical species, which then in turn potentiate the release of excitatory neurotransmitters (i.e.- glutamate) and promote NMDA receptor stimulation that both mediate increased NO generation. See, e.g. Eliasson, M.J.L., et al. (1999), "Neuronal Nitric Oxide Synthase Activation and Peroxynitrite Formation in Ischemic Stroke Linked to Neural Damage," Journal of Neuroscience 19(14):5910-5918; and Ghigo, D., et al. (1998), "Chloroquine stimulates nitric oxide synthesis in murine, porcine and human endothelial cells," Journal of Clinical Investigation 102(3): 595-605.

This cyclic generation of damaging species is termed a cytotoxic cascade, which can be precipitated by an ischemic event. If chloroquine is administered following the initiation of the inflammatory response, the promotion of the cytotoxic cascade is potentiated because research shows that I-κB-α is completely degraded within 15 minutes after a noxious event, and chloroquine administration prevents I-κB-α resynthesis. See Chen, F. et al. (1997), "Calpain contributes to silica-induced I kappa B-alpha degradation and nuclear factor-kappa B activation," Archives of Biochemistry & Biophysics 342(2):383-388.

The primary deficit of the '039 patent in failing to enablingly teach one skilled in the art to treat cerebral ischemia with chloroquine, is that one skilled in the art is aware of the impossibility

of administering neuroprotective agents *prior to, immediately prior to* and/or *at the time of* an ischemic event. Pretreating patients for cerebral ischemia is not possible, because these events are unpredictable. Treating patients for cerebral ischemia within less than about ten to fifteen minutes after the event is also not possible because typically patients have not reached a treatment facility within such a short period of time. It is well recognized in the art that in cerebral ischemia, treatment is not undertaken until at least about 6 to about 24 hours after the event. See, e.g., Conference Proceedings, "Stroke Drug Development: Bridging the Gap from Animal Research to Human Trials," March 6-7, 1999, Orlando, Florida, p. 49.

Thus, the only way chloroquine could function as an effective treatment for cerebral ischemia would be to administer it *before* the event, or within about ten to fifteen minutes after the event, i.e. before production of cytokines, TNF-alpha and IL-6 begins. From this it can be seen that when chloroquine is administered before the event, as in the '039 patent, it will prevent neural damage by preventing TNF alpha and IL-6 degradation of I-κB-α. Further it can be seen that administration of more chloroquine after the event and after the patient has been pretreated, as is demonstrated in some of the examples in the '039 patent, will not have any further effect because although it prevents synthesis of more I-κB-α, more I-κB-α is not needed because chloroquine has effectively inhibited the degradation of the I-κB-α which continues to effectively bind to and prevent the activation of inflammatory response activator nuclear factor NF-κB.

To summarize, given before an ischemic event, chloroquine can prevent neural damage. However, the art as a whole teaches that administration of chloroquine for treatment of cerebral ischemia after the first ten minutes will damage the neurons through increased nitric oxide and oxygenated radical production rather than having a protective effect. Subsequently, chloroquine given after an ischemic event would *enhance* neural damage. Therefore the teachings of patent '039 do not enable one skilled in the art, who is aware of the foregoing harmful effects of chloroquine, to treat cerebral ischemia using chloroquine.

Another important reason why the '039 patent fails to enablingly teach the use of chloroquine to treat cerebral ischemia to those skilled in the art of treating ischemia and/or those skilled in the art of administering emergency medical treatment is that those of skill in these arts are aware that chloroquine, administered iv, as is described in the '039 patent, results in cardiovascular toxicity and hypotension. See Scott, V. (1995), "Single intravenous injections of chloroquine in the treatment of falciparum malaria: toxic and immediate therapeutic effects in 11 cases," American Journal of Tropical Medicine and Hygiene 30:701-705; Laing, A. (1955), "The single dose treatment of falciparum malaria with Nivaquine: a review of 164 cases treated at the district hospital Kuala Langsar," Medical Journal of Malaya 9:216-221; Don-Michael, T. and Aiwazzadeh, S. (1970), "The effects of acute chloroquine poisoning with special reference to the heart," American Heart Journal 79:831-842; Sofola, O. (1980) "The cardiovascular affect of chloroquine in anesthetized dogs," Canadian Journal of Physiological Pharmacology 58:836-841. Persons who are skilled in the art of treating a suspected and/or confirmed victim of cerebral ischemia would not administer an agent (such as both the drug and method of drug delivery described in '039), that has a potential to induce cardiovascular toxicity and/or hypotension. It is well known to those skilled in the art of treating cerebral ischemia, that hypotension worsens a

stroke victim's prognosis and that agents capable of inducing a hypotensive state are contraindicated for use in patients who are experiencing an ischemic event.

Further, chloroquine is well known to the art to induce neurological and psychiatric effects such as hallucinations (see, e.g., Physician's Desk Reference, 2000, of record. Medical personnel who treat cerebral ischemia would not consider it reasonable to administer an agent capable of confounding a proper diagnosis by promoting the generation of neurological disturbances or stimulating psychiatric effects, such as are known to occur following chloroquine administration. Again, the '039 patent does not enablingly teach the use of chloroquine for cerebral ischemia to those of skill in the art because such a drug would be contraindicated, especially in cases where a valid neurological evaluation is required to accurately assess the severity of the ischemic event suffered.

The final reason why the '039 patent fails to teach those skilled in the art to use chloroquine to treat ischemia is that the '039 patent employs a defective study design and poor animal models for the experiments they claim demonstrate neuroprotection. Persons skilled in the art of stroke drug development would perceive little, if any, validity in the '039 patent's inference that these drugs would provide neuroprotection to humans who were faced with similar cerebral assaults as the rodents used in the '039 experiments. Several of the more obvious deviations from proper stroke drug development study design, as they appear in the methods discussions in '039, are presented below.

Neural damage, e.g. resulting from a five-minute occlusion, would not be expected to show up until about 7-28 days after cutting off blood flow. See Conference Proceedings, "Stroke Drug Development: Bridging the Gap from Animal Research to Human Trials," March 6-7, 1999, Orlando, Florida, and p. 28. However, in the '039 patent, results were evaluated only 24 hours after the event in rats and 4 to 6 days after the event in gerbils, while the art teaches that neural protective effects seen earlier tend to evaporate – indicating a mere postponement of injury rather than real protection.

Further, rats and gerbils, the animals in which results in the '039 patent were generated, are not good animal models for cerebral ischemia in humans. Gerbils are notorious for false positive results in studies involving neural protection (see Feuerstein, G.Z. and Wang, X (2000), "Animal models of stroke," Molecular Medicine Today 6(March):133-135), and both rats and gerbils are poor models for cerebral ischemia because repercussion injury (which occurs in humans by 24 hours, at about 50%) does not occur in rodents (see Conference Proceedings, "Stroke Drug Development: Bridging the Gap from Animal Research to Human Trials," March 6-7, 1999, Orlando, Florida, pp 20-21). See also, Cockcroft, KM, et al. (1996), "Cerebroprotective Effects of Aminoguanidine in a Rodent Model of Stroke," Stroke 27(8):1393-1398 and Editorial Comment by G. Feuerstein, M.D. at p. 1398, which indicates that a neural protective affect appearing two hours after ischemia did not occur three hours after ischemia.

Moreover, it is my opinion that the patent does not teach or suggest the use of targeting agents with chloroquine for any purpose. A targeting agent would increase the amount of chloroquine reaching the brain, which would intensify the harmful effects discussed above.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Patricia L. Strenahan, M.D. Ph.D

Patricia L. Strenahan, M.D.Ph.D.

Date: Jan. 14, 2002